Abstract

Immunogenic cancer cell death triggered by free and polymer-bound doxorubicin

Water-soluble polymeric drug carriers based on *N*-(2-hydroxypropyl) methacrylamide (HPMA) have been developed to avoid undesirable side-effects of systemically active cytostatic drugs. Conjugates based on HPMA copolymer passively accumulate in tumor tissue of solid tumors due to the effect of enhanced vascular permeability and retention effect (EPR effect). Active accumulation can be achieved by binding of targeting structure recognising tumor-specific receptors to the polymeric carrier. Conjugation of free doxorubicin to HPMA polymeric carrier significantly reduces its nonspecific side effects *in vivo* by maintaining effective antitumor activity *in vitro* and *in vivo*. Besides direct cytotoxic effect on tumor cells, HPMA conjugates with bound doxorubicin possess immunostimulatory properties and induce long-lasting anti-tumor immunity in cured mice. Recent studies have shown that free doxorubicin induce immunogenic apoptosis in tumor cells. Those cells are dying by cell death which possesses characteristic markers of apoptosis but these cells are able to activate the immune system and thus induce effective anti-tumor immune response. This phenomenon has been described as crucial for a development of treatment-induced antitumor immunity. We found that the immunogenic characteristics of apoptosis are present only in cells exposed to free doxorubicin or conjugate which doxorubicin bound *via* hydrasone, pH-sensitive bond. By contrast, conjugate with doxorubicin bound to HPMA carrier via enzymatically degradable, amide bond does not induce immunogenic apoptosis in tumor cells. However, in *in vivo* experiments are both types of conjugates very effective inducers of antitumor immunity. Furthermore, we observed that cells were more phagocytosed by dendritic cells when incubated with both free or HPMA copolymer bound doxorubicin. That means that both types of HPMA conjugates are able to stimulate antitumor immunity, although only the conjugate with doxorubicin bound pH-sensitive bond can induce immunogenic apoptosis. On the other hand conjugate containing amide bound doxorubicin changes saccharide composition of surface glycoproteins on cancer cells. Cells incubated with this conjugate express increasing level of CD43 molecule, providing a binding site for galectin-1, which triggers cell apoptosis in tumor cell. Thus we have also shown that cells exposed to conjugates with amide bound doxorubicin are more sensitive to apoptosis induced by galectin-1 than the cells exposed to free or hydrazone bound doxorubicin.